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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/GB99/00876 (22) International Filing Date: 19 March 1999 (19.03.99) (30) Priority Data: 9805913.2 19 March 1998 (19.03.98) GB		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
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(54) Title: DIAGNOSIS OF SPONGIFORM OR DE-MYELINATING DISEASE			
(57) Abstract			
<p>A method for detecting a de-myelinating disease or spongiform encephalopathy in mammals comprises testing a biological sample obtained from the mammal for IgA antibodies indicative of infection by an <i>Acinetobacter</i> species. The <i>Acinetobacter</i> species is one which presents to the mammal an antigen which exhibits molecular mimicry with the myelin of the mammal e.g. <i>Acinetobacter calcoaceticus</i>. The antibodies tested for are antibodies which bind to an epitope present in or derived from the <i>Acinetobacter</i> species or to a prepared peptide sequence corresponding thereto or to a conformationally similar peptide sequence e.g. the peptide sequence RFSAWGAE or ISRFAWGEV. The method tests for bovine spongiform encephalopathy, multiple sclerosis and Creutzfeldt-Jacob disease in humans. A test kit uses as the test antigen the whole <i>Acinetobacter</i> organism or at least one prepared peptide sequence as described above and a secondary antibody against the human, bovine, or other mammalian IgA.</p>			

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DIAGNOSIS OF SPONGIFORM OR DE-MYELINATING DISEASE

This invention relates to the diagnosis of de-myelinating diseases and spongiform encephalopathies in animals and humans.

In our copending application WO 98/13694 we have disclosed a new diagnostic test for spongiform encephalopathies and other de-myelinating conditions in mammals. The test disclosed in our prior application is based on a model of the genesis of this pathological state which is applicable to the various forms in which it is manifest in humans and animals. In relation to the bovine spongiform disease this model provides an alternative to the current theory based on the formation of prions. Briefly, this new model is based on the phenomenon of molecular mimicry according to which mammals exposed to certain bacteria having peptide sequences which mimic myelin peptides experience an auto-immune reaction. In our prior application we indicated that human de-myelinating diseases were also open to the same explanation according to our new model disclosed therein.

According to the present invention, a method for detecting a de-myelinating disease or spongiform encephalopathy in mammals comprises testing a biological sample obtained from the mammal for IgA antibodies indicative of infection by an *Acinetobacter* species. We believe that infective micro-organisms of these species present to the mammal an antigen which exhibits molecular mimicry with the myelin of the mammal. The phenomenon of molecular mimicry has been explained in our above-mentioned prior

application WO 98/13694, the contents of which are hereby incorporated by reference.

We have now confirmed the presence of elevated levels of certain antibodies in human sera of patients suffering from multiple sclerosis (MS). These are the IgA antibodies to *Acinetobacter* species e.g. *Acinetobacter calcoaceticus*, the same organisms for which antibodies were previously found in BSE sera. Similar results have been obtained for Creutzfeldt-Jakob disease (CJD). Tests for antibodies in sera from patients who had died of CJD also show increased levels, this being especially marked for the IgA antibody sub-class. The same IgA specificity also applies to bovine sera used for the tests described in our above-mentioned copending application.

It is clear that humans suffering from MS and CJD and cows suffering from BSE all have very significantly raised levels of *Acinetobacter calcoaceticus* IgA antibodies in their blood. Tests for such antibodies in sera from living subjects at an early stage make it possible to identify those liable to develop these diseases. The present invention opens up the opportunity of early treatment of these infections e.g. by use of an appropriate antibiotic to prevent further autoimmune attack on the subjects' own myelin.

As also indicated in our application WO 98/13694, *Acinetobacter calcoaceticus* is one species of *Acinetobacter* which provides an antigen which stimulates the formation of antibodies which cross-react with the mammalian myelin . Antibodies have been demonstrated to react with several strains of this species including 17905, AC606, SP13TV, 105/85, and 11171. These strains are in the

Reference Centre for *Acinetobacter* species held by Dr Kevin Towner, Public Health Laboratory, University of Nottingham, U.K.

In carrying out the present invention, the test is for antibodies which bind to an epitope present in or derived from the *Acinetobacter* species. The antigen used in the test may be the whole organism or at least one prepared peptide sequence corresponding to an *Acinetobacter* epitope. Alternatively, peptide sequences may be used which have minor variations in amino-acid sequence from the above-mentioned epitopes or prepared peptides but are conformationally sufficiently similar to them that they also bind to the relevant antibodies. For example, peptides having the sequence RFSAWGAE or ISRFAWGEV may be used.

A test kit for use according to the invention therefore contains at least one test antigen as just indicated. In order to reveal IgA antibodies the kit also contains a secondary antibody against the human, bovine, or other mammalian IgA.

As indicated in WO 98/13694, antibodies are assayed and a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

In view of the greater specificity of the IgA antibodies in the immune response it may be concluded that the mechanism of infection with *Acinetobacter* is via the mucous membranes of the body, the primary sites being the gut or the nasal passages. Since a further correlation has been observed between MS sufferers and patients with major sinus infections, it is probable that the nasal passages

EXAMPLE

The assay for the above mentioned organisms is described in our co-pending application mentioned above. The improved method used herein is as follows:-

ELISA TEST

- 1) Aliquots of 200 ul of the diluted suspension of *Acinetobacter calcoaceticus* (NCIMB 10694, Aberdeen) grown in nutrient broth are absorbed onto 96 well flat bottomed rigid polystyrene microtitre plates overnight at 4°C.
- 2) The plates are then washed 3 times with phosphate buffered saline (PBS), 0.1% (v/v) Tween 20.
- 3) Aliquots of 200 µl of blocking solution (0.2% w/v ovalbumin, 0.1% v/v Tween 200 in PBS is added to each well and incubated for one hour at 37°C.
- 4) The plates are then washed 3 times with PBS.Tween 20.
- 5) Aliquots of 200 µl serum samples (test or control) diluted 1/200 in PBS. Tween 20 is added and incubated for 2 hours at 37°C.
6. The plates are then washed 3 times with PBS.Tween 20.
- 7) Aliquots of 200 µl of peroxidase conjugated rabbit anti-human IgA or rabbit anti-cow IgA , diluted 1/4000 (cow) (or 1/500 for human) with PBS.Tween 20 are added and incubated for 2 hours at 37°C.
- 8) The plates are then washed 3 times with PBS.Tween 20.

9) The development of the colorimetric assay takes place at room temperature for 20 minutes, after the addition of 200 µl per well of 0.5 mg/ml (2,2'-azinobis(3-ethylbenz-thiazoline-6-sulphonic acid) in citrate/phosphate buffer, pH 4.1, containing 0.98 mM hydrogen peroxide.

10) the reaction is then stopped with 100 µl of 2 mg/ml sodium fluoride and optical densities measured at a wavelength of 630 nm with a micro-ELISA plate reader.

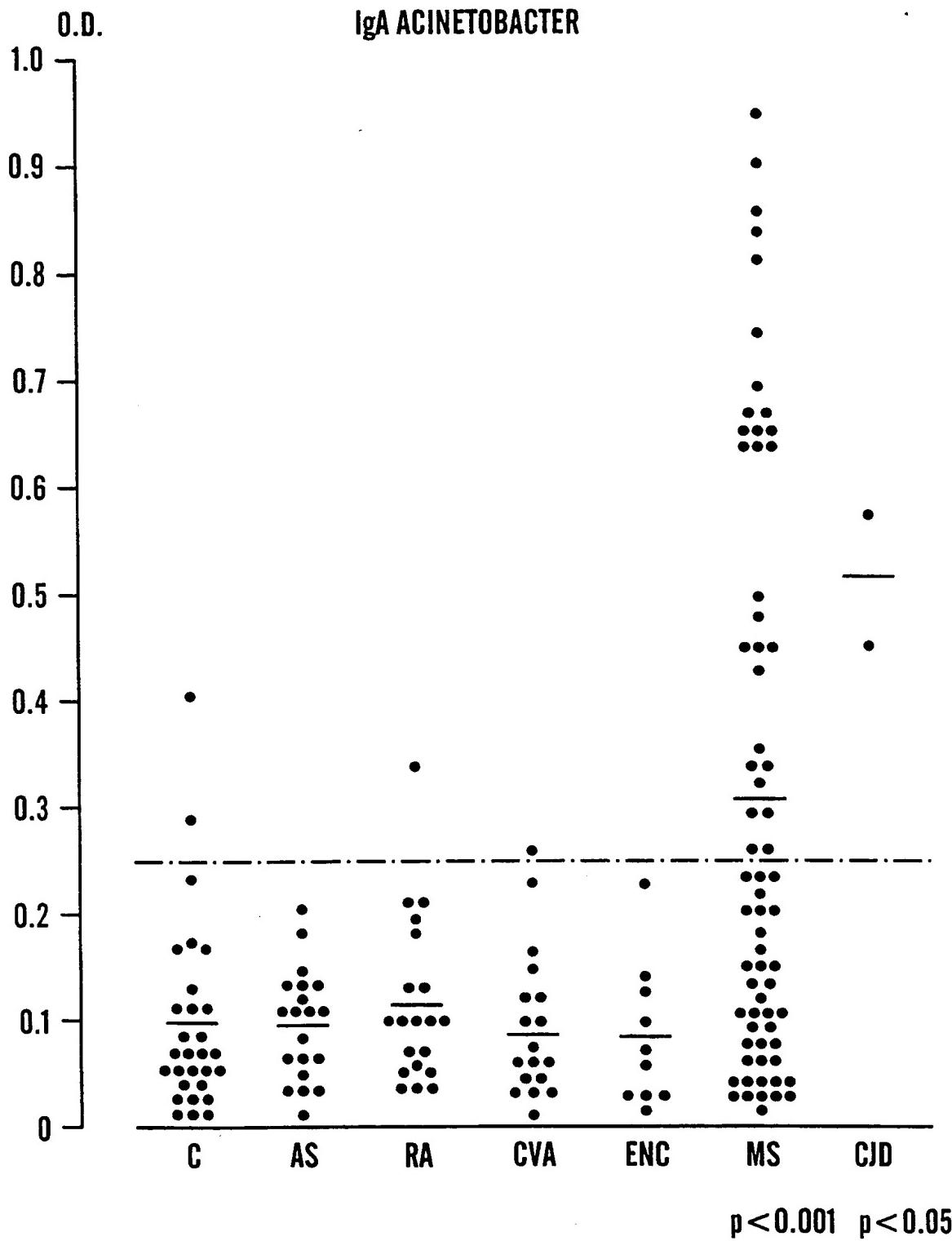
Results for MS and CJD are shown in the attached Figure 1 and those for BSE are shown in Figure 2. These give the titres of IGA *Acinetobacter* antibodies in MS and CJD sera, BSE sera, and control sera. The dashed line represents the 95% confidence limits of the controls.

CLAIMS

1. A method for detecting a de-myelinating disease or spongiform encephalopathy in mammals which comprises testing a biological sample obtained from the mammal for IgA antibodies indicative of infection by an *Acinetobacter* species.
2. A method according to claim 1, in which the *Acinetobacter* species is one which presents to the mammal an antigen which exhibits molecular mimicry with the myelin of the mammal.
3. A method according to claim 1 or 2, in which the antibodies are indicative of prior infection by *Acinetobacter calcoaceticus*.
4. A method according to claim 1, 2, or 3, in which the antibodies tested for are antibodies which bind to an epitope present in or derived from the *Acinetobacter* species or to a prepared peptide sequence corresponding thereto or to a conformationally similar peptide sequence.
5. A method according to claim 4, in which the epitope contains the peptide sequence RFSAWGAE.
6. A method according to claim 4, in which the epitope is the peptide sequence ISRFAWGEV.

7. A method according to any of claims 1 to 6, in which the disease tested for is bovine spongiform encephalopathy.
8. A method according to any of claims 1 to 6, in which the disease tested for is multiple sclerosis in humans.
9. A method according to any of claims 1 to 6, in which the disease tested for is Creutzfeldt-Jacob disease in humans.
10. A method according to any of the preceding claims in which antibodies are assayed and a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.
11. A test kit for use with a method according to any of the preceding claims, in which the test antigen is the whole *Acinetobacter* organism or at least one prepared peptide sequence corresponding to an *Acinetobacter* epitope or a variant peptide sequence which is conformationally sufficiently similar to it to bind to the relevant antibodies, and a secondary antibody against the human, bovine, or other mammalian IgA.
12. A test kit according to claim 11, comprising a peptide having the sequence RFSAWGAE or ISRFAWGEV.
13. A test kit according to claim 11 or 12, in which the secondary antibody is a rabbit anti-human IgA or rabbit anti-bovine IgA.

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LEGEND: IgA ANTIBODIES TO ACINETOBACTER BACTERIA, MEASURED BY ELISA IN HEALTHY CONTROLS (C) AND PATIENTS WITH ANKYLOSING SPONDYLITIS (AS), RHEUMATOID ARTHRITIS (RA), CEREBRO-VASCULAR ACCIDENTS (CVA), VIRAL ENCEPHALITIS (ENC), MULTIPLE SCLEROSIS (MS) AND CREUTZFELDT-JAKOB DISEASE (CJD). (p-VALUES INDICATE SIGNIFICANCE COMPARED TO CONTROLS)

Fig. 1

SUBSTITUTE SHEET (RULE 26)

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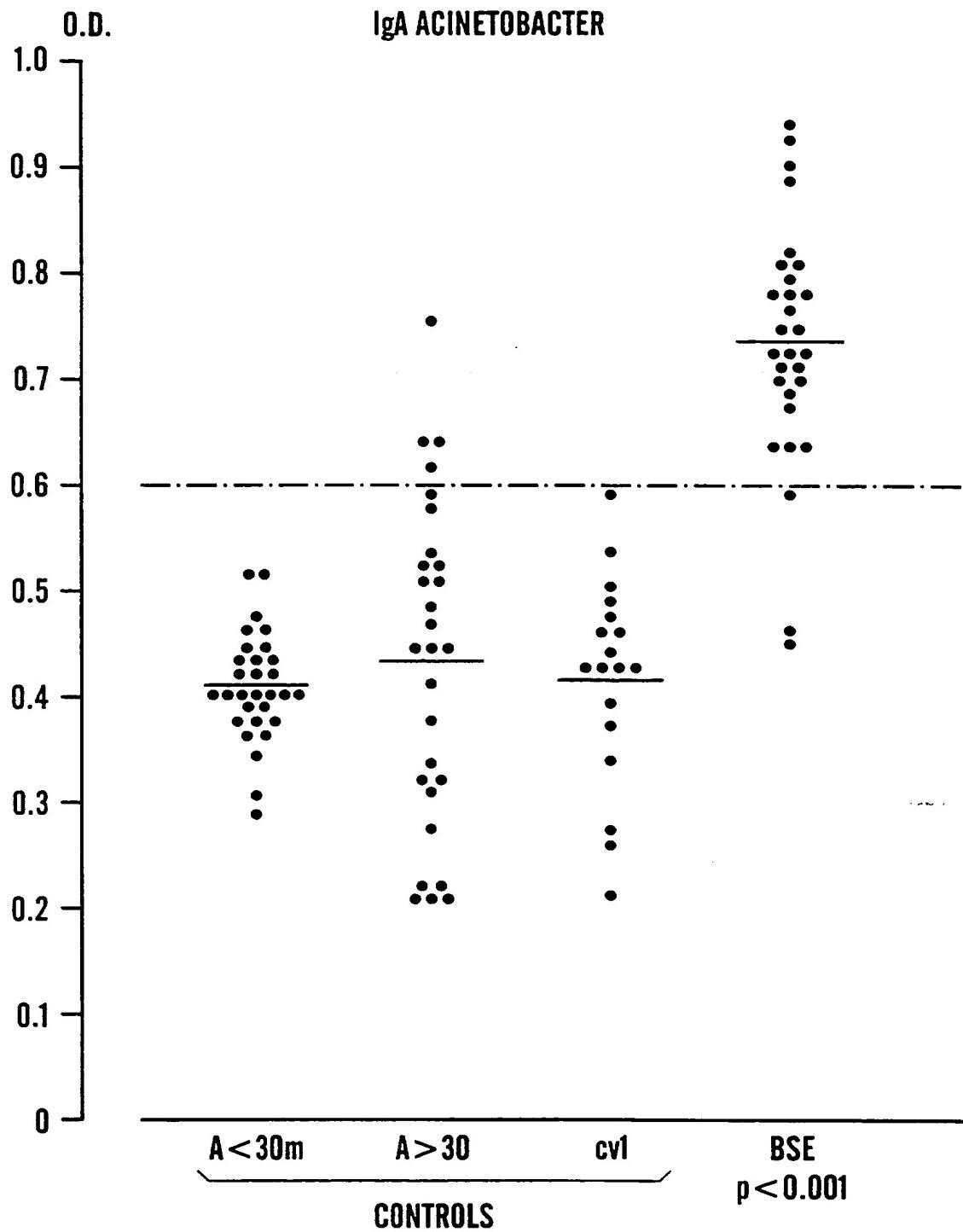
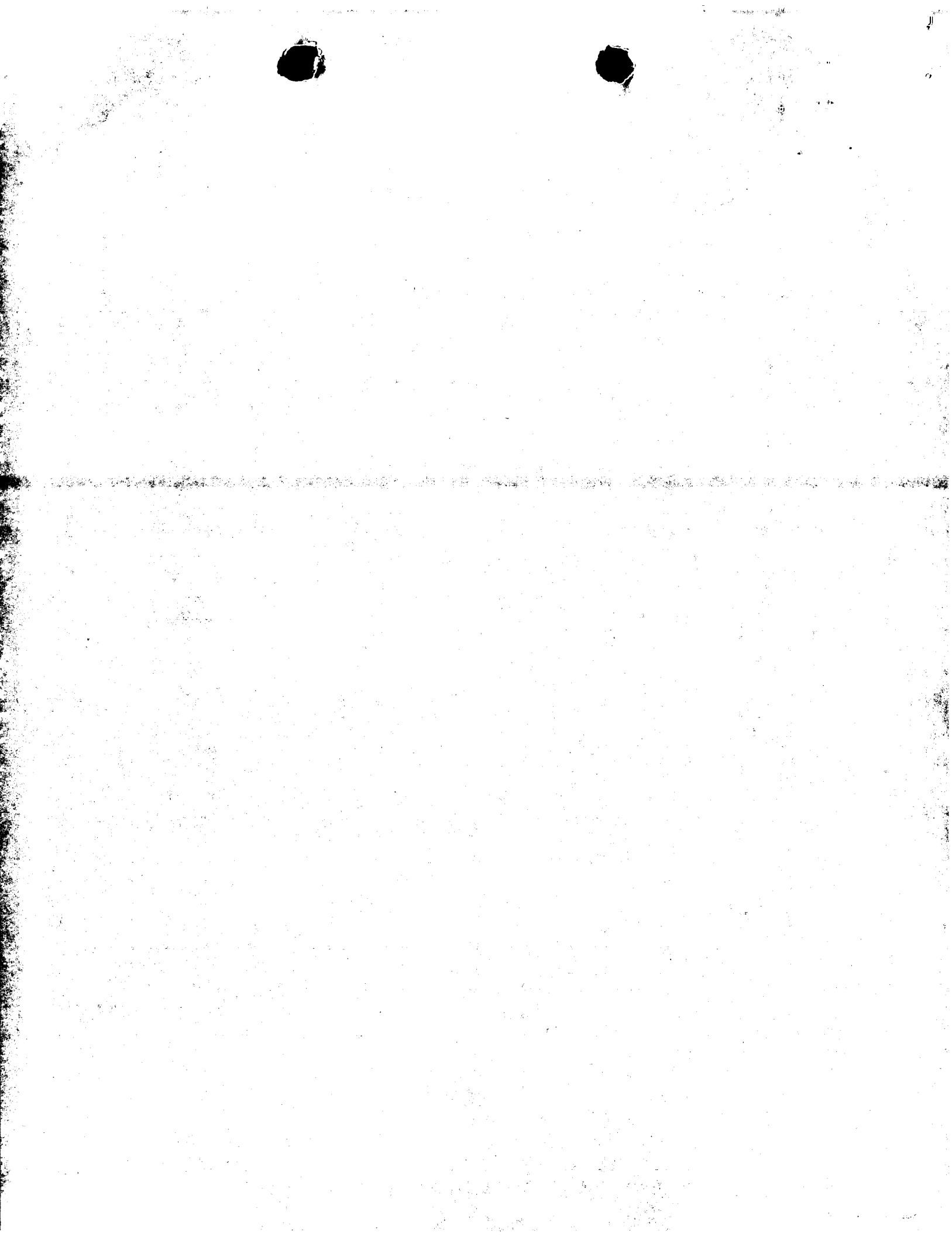


Fig.2



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(21) International Application Number: PCT/GB99/00876		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
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(71) Applicant (<i>for all designated States except US</i>): KING'S COLLEGE, UNIVERSITY OF LONDON [GB/GB]; The Strand, London WC2R 2LS (GB).			
(72) Inventor; and		Published	
(75) Inventor/Applicant (<i>for US only</i>): EBRINGER, Alan [GB/GB]; 76 Gordon Road, Ealing, London W5 2AR (GB).		With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
(74) Agents: POWELL, Stephen, David et al.; Williams, Powell & Associates, 4 St. Paul's Churchyard, London EC4M 8AY (GB).		(88) Date of publication of the international search report: 11 November 1999 (11.11.99)	

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(57) Abstract

A method for detecting a de-myelinating disease or spongiform encephalopathy in mammals comprises testing a biological sample obtained from the mammal for IgA antibodies indicative of infection by an *Acinetobacter* species. The *Acinetobacter* species is one which presents to the mammal an antigen which exhibits molecular mimicry with the myelin of the mammal e.g. *Acinetobacter calcoaceticus*. The antibodies tested for are antibodies which bind to an epitope present in or derived from the *Acinetobacter* species or to a prepared peptide sequence corresponding thereto or to a conformationally similar peptide sequence e.g. the peptide sequence RFSAWGAE or ISRFAWGEV. The method tests for bovine spongiform encephalopathy, multiple sclerosis and Creutzfeldt-Jacob disease in humans. A test kit uses as the test antigen the whole *Acinetobacter* organism or at least one prepared peptide sequence as described above and a secondary antibody against the human, bovine, or other mammalian IgA.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/00876

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CHEMICAL ABSTRACTS, vol. 80, no. 11, 18 March 1974 (1974-03-18) Columbus, Ohio, US; abstract no. 56313, A. WAJGT.: "Assessment by immunofluorescence methods of humoral antimyelin antibody in rats with cyanide encephalopathy." page 68; column 1; XP002052988 abstract & ANN. IMMUNOL. (POZNAN), vol. 5, no. 1-2, 1973, pages 51-58, ---</p> <p style="text-align: center;">-/-</p>	1

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

22 September 1999

Date of mailing of the international search report

30/09/1999

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	B. H. TOH ET AL.: "The 200- and 150-kDa neurofilament proteins react with IgG autoantibodies from patients with kuru, Creutzfeldt-Jakob disease, and other neurologic diseases." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 82, May 1985 (1985-05), pages 3485-3489, XP002052986 WASHINGTON US ---	
A	R. L. SIDMAN ET AL.: "Transmissible spongiform encephalopathy in the gray tremor mutant mouse." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 82, January 1985 (1985-01), pages 253-257, XP002052987 WASHINGTON US ---	
A	CHEMICAL ABSTRACTS, vol. 109, no. 21, 21 November 1988 (1988-11-21) Columbus, Ohio, US; abstract no. 187890. M. P. MCKINLEY ET AL.: "Developmental regulation of prion protein mRNA in brain." page 484; column 2; XP002052989 abstract & CIBA FOUND. SYMP., vol. 135(Novel Infect. Agents Cent. Nerv. Syst.), 1988, pages 101-116, ---	
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9813694 A	02-04-1998	EP 0929813 A	21-07-1999

